Ordinary Differential Equations for Modelling Bacterial Interactions in the Gut

Wilkinson, Michael H.F.

Model Intestinal Microflora In Computer Simulation (MIMICS) Technical Report:

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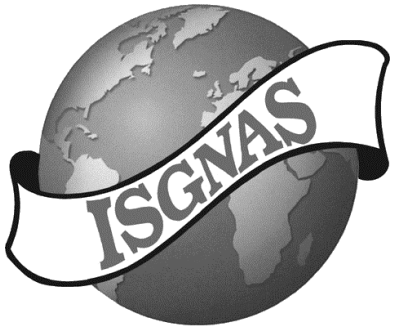
Michael H.F. Wilkinson
Centre for High Performance Computing
University of Groningen
Landleven 1, 9747 AD Groningen, The Netherlands
Phone: +31-50-363-3374
Fax: +31-50-363-3406
E-mail: michael@rc.service.rug.nl



MIMICS Technical Reports

The MIMICS project of the Centre for High Performance Computing of the University of Groningen is a project initiated by the International Study Group for New Antimicrobial Strategies (ISGNAS). Its aim is to explore computer simulation methods for the study of the intestinal microflora and its interactions with the host. MIMICS technical reports are intended to explain various technical issues involved in this modelling. As such, the main readership are persons involved in the MIMICS project, other ISGNAS projects, and those intending to implement similar models. Parts of the contents may be reproduced in articles at a later date.

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1. Introduction

In any complex microbial ecosystem, such as the gut microflora, many different types of interactions can take place. In this paper I propose a classification of interactions, and discuss the appropriate ordinary differential equations belonging to each class. The steady state behaviour of each class is considered in the binary case, i.e. interaction between just two species. Stability analysis and dynamic behaviour are discussed in a number of cases. It is also shown that similarities exist between interbacterial and host/bacterium interactions, and that the mathematical behaviour of these interactions should be the same qualitatively. Modelling can therefore be simplified, since the same equations can be used for two types of interactions.

The classification of interactions is as follows:

1. **Pure food competition.** Two species may compete for the same food source which is readily available to either bacterium in the absence of the other.
2. **Parasitic food competition.** If one species produces extracellular enzymes which lyse macromolecules, other species may compete for the reaction products.
3. **Symbiotic food interactions.** One species may use the metabolites produces by another as a limiting substrate. If the metabolites are inhibitory to the latter species, the interaction becomes mutualistic.
4. **Toxin production.** Bacteria may produce toxins (bacteriocins) which kill or inhibit others. Production of inhibitory metabolites is modelled in the same way. May lead to multiple stable equilibria, and hence to irreversibility of changes in the intestinal microflora.
5. **Toxin inactivation.** Resistant bacteria may remove or inactivate toxins, either by use in their metabolisms, or by secretion of inactivating substances, protecting sensitive species.
6. **Predator-prey interaction.** One species may prey on another (e.g. *Bdellovibrio*, *Daptobacter*).
7. **Decoys for predators or phages.** Bacteria resistant to phages or attack by predators may act as decoys, reducing the effect on host or prey species by competitive inhibition.

8. **Direct binding site competition.** Bacteria may compete for binding sites on the intestinal epithelium. Competition may be through increased motility or chemotaxis, which increase the number of collisions with the wall. Tighter binding by increased affinity is an alternative mechanism.
9. **Indirect binding site competition.** Bacteria may produce substances which reduce the affinity of other bacteria for wall sites. Lectins are suitable examples for this type of interaction.
10. **Biofilm gel production.** Bacteria may secrete extracellular polymers which stabilize the biofilm on the epithelium. Bacteria embedded in this mucus layer need not strictly be bound to the epithelium, yet they are protected from sloughing.
11. **Biofilm gel destruction.** Bacteria may lyse extracellular polymers. Active destruction of the biofilm gel is sometimes used by bacteria to dissociate from the surface when food is scarce. Bacteria which bind tightly to the epithelium itself may use this method to rid themselves from competition of gel-embedded competitors.
12. **"Meta-adherence."** Bacteria may bind to other bacteria already bound to the epithelium. One example is the frequent observation of small rods or cocci bound to SFBs.
13. "**Quorum sensing**" and other "pheromone" mediated interactions. A comparatively recent discovery is the ability of some bacteria to secrete substances which regulate gene expression at the population level.
14. **"Pathway clearing by copiotrophs."** Bacteria may remove toxic levels of substrate, allowing obligate oligotrophs to colonize. Once established, they may outcompete the initial copiotrophs by further lowering of substrate levels.

Differential equations for each of these interactions, except 9 through 11 and 13 have been drawn up. Many of these interactions also apply to the host-bacterium system:

1. **Pure food competition.** Host and bacteria compete for readily available resources such as monosaccharides, amino acids, etc.
2. **Parasitic food competition.** The host may produce enzymes which lyse macromolecules, yielding products for which a non-producing bacterium competes parasitically. The reverse may also be the case (i.e., the bacterium is the enzyme producer).

3. **Symbiotic food interactions.** Metabolites of the host may be used by bacteria, and vice-versa (cf. short chain fatty acid use by enterocytes).
4. **Toxin production.** Bacterial toxins may harm the host, and the host may produce toxins to kill bacteria.
5. **Toxin inactivation.** Bacteria may remove or inactivate substances toxic to the host.
6. **Predator-prey interaction.** Macrophages, etc., may be considered predators in the mucosa.
7. **Indirect binding site competition.** The host may produce substances which reduce the affinity of bacteria for wall sites.
8. **Biofilm gel production.** The host may secrete polymers which stabilize the biofilm on the epithelium.
9. **Biofilm gel destruction.** The host may lyse polymers in the mucus, to actively remove bacteria.

The reason for noting the equivalence between inter-bacterial interactions and host-microflora interactions is that *the same* differential equations govern the behaviour of equivalent interactions. Therefore, the same, or at least very similar programming techniques can be used to simulate the two classes of interaction.
Differential equations for most of these interactions are drawn up in the following sections, after a brief description of models of elementary reactions. The further discussion assumes the reader is understands some of the basics of ordinary differential equations, and the principles of stability analysis. Others may wish to consult one of many textbooks on mathematical methods in (microbial) ecology [DeAngelis, 1992; Koch *et al.* 1997].

2. The Basic Components of Interactions

Most interactions can be built from a limited number of components: substrate uptake, enzyme activity, inhibition and activation of reactions (metabolic pathways), secretion of substances, and transport components. These components are described in the following subsections.

2.1 Enzyme activity, inhibition, and activation.

The celebrated Michaelis-Menten equation for the reaction rate *v* for an enzyme *E* acting on single substrate (*S*) molecules is

$$V(E,S)=V_{max}\frac{ES}{K_S+S}, \tag{1}$$

in which V_{max} is the maximum reaction rate per unit of enzyme and K_S is the saturation or Michaelis-Menten constant. A simple generalization for reactions of *n* molecules of substrate is

$$V(E,S)=V_{max}\frac{ES^n}{K_S+S^n}. \tag{2}$$

Many enzymes have sites to which other substances may adhere, causing activation or non-competitive inhibition of the enzyme. If an enzyme has *n* such sites, equation (1) becomes

$$V(E,S)=V_{max}\frac{ES}{K_S+S}\frac{K_{inh}}{K_{inh}+I^n}, \tag{3a}$$

$$V(E,S)=V_{max}\frac{ES}{K_S+S}\frac{A^n}{K_{act}+A^n}, \tag{3b}$$

for inhibition by *I* and activation by *A* respectively.

Alternatively, substances may adhere to the active site, blocking the action of the enzyme, causing competitive inhibition. In that case, (1) becomes

$$V(E,S)=V_{max}\frac{ES}{K_S+S+K_{inh}I}. \tag{4}$$

If sufficient substrate is present, the maximum reaction rate may still be attained, unlike the case of non-competitive inhibition.

2.2 Substrate uptake

2.2.1 Single substrate uptake

Substrate uptake and growth are usually modelled using the Michaelis-Menten equation. It is assumed that a single rate-limiting step determines the uptake and growth rate. In that case, the uptake rate can be modelled through (1), with $v_{\max}$ the maximum specific uptake rate. The equation for growth of a bacterium *X* becomes

$$\frac{\partial X}{\partial t} = Y_S V_{max} \frac{S}{K_S + S} X = \mu_{max} \frac{S}{K_S + S} X \,, \tag{5}$$

where Y_S is the yield of bacterial biomass per unit of substrate, and $\mu_{max} \equiv Y_S V_{max}$ is the maximum specific growth rate. At low substrate concentrations, (5) may be approximated by

$$\frac{\partial X}{\partial t} = \frac{\mu_{max}}{K_S} SX = a_S^0 SX \,, \quad \text{or} \quad a_S^0 \equiv \frac{1}{X} \frac{\partial}{\partial S} \frac{\partial X}{\partial t}\bigg|_{S=0} = \frac{\mu_{max}}{K_S}, \tag{6}$$

with a_S^0 the specific affinity. Equation (5) is usually referred to as the Monod equation. However, in the case of predator-prey systems, where *S* now denotes the prey species, (5) is referred to as the Holling type II equation.

Many other equations may be used instead of the Monod (Holling II) equation. All need to share two features: (i) substrate limited growth at low substrate concentrations, and (ii) saturation of growth rate at high substrate concentrations, due to internal limitations. An early, purely heuristic model is that of Blackman [1905], which has two linear branches, and a discontinuous first derivative where the two branches meet:

$$\mu(S) = \begin{cases} a_S^0 S & S < K_S \\ \mu_{\max} & S \geq K_S \end{cases}. \tag{7}$$

Blackman's equation is problematic for two reasons: (i) it does not model real growth very well, and (ii) it is computationally inefficient (comparisons are costly).

A more interesting alternative is the model by Best [1955], which assumes that passive diffusion of substrate limits growth. Use of substrate in the cell through a Michaelis-Menten type irreversible enzyme mediated reaction creates a concentration gradient which maintains the influx of substrate through diffusion. Best's equation for growth rate is

$$\mu(S) = \mu_{max} \frac{S + K_S + J}{2J} \left(1 - \sqrt{1 - \frac{4SJ}{(S + K_S + J)^2}} \right), \tag{8a}$$

and

$$a_S^0 \equiv \frac{1}{X} \frac{\partial}{\partial S} \frac{\partial X}{\partial t} \bigg|_{S=0} = \frac{\mu_{max}}{K_S + J} \tag{8b}$$

in which J is a parameter for diffusion through the cell wall. If diffusion is slow (J is large) (8a) converges on the Blackman model, for very high diffusion rates the model converges on the Monod case [Koch, 1997]. Best's model was largely ignored after the discovery of transport proteins, since these supported Monod's model. However, Koch and Wang [1982] have shown that diffusion through porins in the outer membrane may actually be a rate limiting factor.

2.2.2 Multiple substrate uptake

Multiple substrate uptake can take two distinct forms: (i) uptake of substances which perform different tasks within the cell (e.g. sources of carbon, nitrogen or phosphorus), and (ii) uptake of different substrates which can essentially replace each other (e.g. glucose and lactose can both be used as carbon source for *Escherichia coli*). In the first case, the uptake terms can be either *interactive* or *non-interactive*. Interactive terms all limit growth to a certain extent. Each substrate may be thought of as activator for each other substrate (as in eq. (3b)). For two substrates, the interactive case is modelled as

$$\mu(S_1, S_2) \equiv \frac{1}{X} \frac{\partial X}{\partial t} = \mu_{max} \frac{S_1}{K_{S,1} + S_1} \frac{S_2}{K_{S,2} + S_2}, \tag{9}$$

which can be generalized to N substrates as

$$\mu(S_1, S_2, \ldots, S_N) = \mu_{max} \prod_{i=1}^{N} \frac{S_i}{K_{S,i} + S_i}. \tag{10}$$

In the non-interactive case, only one substrate is limiting, i.e., the substrate in shortest supply relative to growth requirements dictates the speed of growth. The non-interactive case for two substrates becomes

$$\mu(S_1, S_2) = \mu_{max} \min\left(\frac{S_1}{K_{S,1} + S_1}, \frac{S_2}{K_{S,2} + S_2} \right), \tag{11}$$

which can be generalized to *N* substrates as

$$\mu(S_1, S_2, \ldots, S_N) = \mu_{max} \min_{i=1}^{N} \frac{S_i}{K_{S,i} + S_i}. \tag{12}$$

If multiple substrates may be used for a similar function (e.g., carbon source), the growth terms are simply a sum of different Monod terms

$$\mu(S_1, S_2) = \mu_{max,1} \frac{S_1}{K_{S,1} + S_1} + \mu_{max,2} \frac{S_2}{K_{S,2} + S_2}, \tag{13}$$

or more generally

$$\mu(S_1, S_2, \ldots, S_N) = \sum_{i=1}^{N} \mu_{max,i} \frac{S_i}{K_{S,i} + S_i}. \tag{14}$$

A further generalization is to allow the bacterium to adjust each maximum specific growth rate $\mu_{max,i}$ as a function of substrate availability

$$\mu(S_1, S_2, \ldots, S_N) = \sum_{i=1}^{N} \mu_{max,i}(S_1, S_2, \ldots, S_N) \frac{S_i}{K_{S,i} + S_i}. \tag{15}$$

To ensure realism, the sum of all maximum *specific* growth rates must never exceed some sum total maximum growth rate

$$\sum_{i=1}^{N} \mu_{max,i}(S_1, S_2, \ldots, S_N) \leq \mu_{max}. \quad \text{for all values of } S_1, S_2, \ldots, S_N. \tag{16}$$

2.3 Secretion

Two forms of secretion may be recognized: (i) secretion as a result of the basal metabolism of bacteria, and (ii) secretion of metabolic by-products of the uptake of substrate *S*. The first case is the simplest to model. Suppose that bacterium *X* secretes a substance *M* at a rate ν_M per unit of bacterial biomass. The rate of production is then simply

$$\frac{\partial M}{\partial t} = \nu_M X, \tag{17a}$$

and the growth rate of the bacterium must then be adapted to

$$\frac{\partial X}{\partial t} = (\mu(S) - \mu_M)X, \tag{17b}$$

in which μ_M is the part of the basal metabolism responsible for production of *M*. It is possible to define a yield of *M* per unit of biomass lost to *X* as $Y_M = \nu_M / \mu_M$.

In the second case, the rate of production of *M* is proportional to the uptake rate. Defining a yield Y_M of metabolite *M* per unit of substrate *S* used, we arrive at

$$\frac{\partial M}{\partial t} = Y_M \nu(S) X = Y_M \frac{\mu(S)}{Y_S} X. \tag{18}$$

The growth rate $\mu(S)$ or uptake rate $\nu(S)$ can take any of the forms described in the previous subsection.

2.4 Transport terms

In large scale models such as the MIMICS V.8 cellular automaton, modelling of transport terms is done by highly complex routines which model both bulk flow and diffusion. The important difference between transport and reaction terms is that transport terms should neither source nor sink any of the substances involved, except material leaving or entering the system through the boundaries. Analytical treatment of such systems is not straightforward, so the transport terms are often simplified to the case of a well-mixed chemostat, with dilution rate D and a constant inflow of nutrients at a fixed concentration S_{in}. The rate of change due to inflow of nutrients is

$$\frac{\partial S}{\partial t} = DS_{in}, \tag{19a}$$

whereas rate of change is S due to the outflow

$$\frac{\partial S}{\partial t} = -DS, \tag{19b}$$

where S is the instantaneous concentration of substrate in the chemostat.

3. The Interactions Proper

3.1 Pure food competition.

Two species compete for the same limiting food source which is readily available to either bacterium in the absence of the other. This is the simplest case, and well studied both in theory and in (chemostat) experiments. It can be modelled using the Monod formalism. Maximum specific growth rate, specific affinity and flow rate parameters determine the outcome of this competition. Only one stable equilibrium in steady state chemostat type ecosystem. When spatial extent is used in the model, motility and chemotaxis are also important.

Determining which species survives in a chemostat model with pure food competition is easy. The differential equation governing growth of a single species X on substrate S is

$$\frac{\partial S}{\partial t} = D\left(S_{in} - S\right) - v(S)X\,, \tag{20a}$$

$$\frac{\partial X}{\partial t} = Y_S v(S)X - DX\,. \tag{20b}$$

Solving for steady state we find

$$X_{eq} = Y_S\left(S_{in} - S_{eq}\right), \tag{21a}$$

$$v\left(S_{eq}\right) = \frac{D}{Y_S}, \quad \text{or} \quad S_{eq} = v^{-1}\left(D/Y_S\right), \tag{21b}$$

provided v^{-1} , which is the inverse of *v*, exists for an uptake rate of D/Y_S . If multiple species compete for the same substrate, the species that has the lowest equilibrium substrate level S_{eq} will outcompete others.

3.2 Parasitic food competition.

Suppose we have fibre (*F*) digesting bacteria (*X*) which produce an external enzyme *E* which degrades the fibre to usable substrate *S*, which is taken up by the bacteria. If these bacteria are growing in a chemostat with dilution rate *D* we can model them using the following set of differential equations:

$$\frac{\partial F}{\partial t} = D\left(F_{in} - F\right) - \frac{v_1 EF}{K_F + F} \tag{22a}$$

$$\frac{\partial S}{\partial t} = \frac{v_1 EF}{K_F + F} - \frac{v_2 XS}{K_S + S} - DS \tag{22b}$$

$$\frac{\partial X}{\partial t} = Y_s \frac{v_2 XS}{K_S + S} - DX - v_3 X \tag{22c}$$

$$\frac{\partial E}{\partial t} = -DE + v_3 X \tag{22d}$$

Solving for steady state we find:

$$E_{eq} = \frac{\nu_3 X_{eq}}{D} \tag{23a}$$

$$S_{eq} = \frac{D+\nu_3}{Y_s \nu_2 - D - \nu_3} K_S \tag{23b}$$

$$F_{eq} = \frac{F_{in} - K_F - \frac{\nu_1 \nu_3 Y_S}{D(D+\nu_3)} \pm \sqrt{\left(F_{in} - K_F - \frac{\nu_1 \nu_3 Y_S}{D(D+\nu_3)}\right)^2 + 4F_{in}K_F\left(1 - \frac{\nu_1 \nu_3 Y_S (F_{in} - S_{eq})}{D(D+\nu_3)}\right)}}{2\left(1 - \frac{\nu_1 \nu_3 Y_S (F_{in} - S_{eq})}{D(D+\nu_3)}\right)} \tag{23c}$$

$$X_{eq} = Y_s \frac{F_{in} - F_{eq} - S_{eq}}{1 + \nu_3 / D} \tag{23d}$$

At equilibrium eigenvalue equation is:

$$\begin{vmatrix} -D - \frac{\nu_1 K_F E}{(K_F+F)^2} - \lambda & \frac{\nu_1 K_F E}{(K_F+F)^2} & 0 & 0 \\ 0 & -D - \frac{\nu_2 K_S X}{(K_S+S)^2} - \lambda & \frac{Y_S \nu_2 K_S X}{(K_S+S)^2} & 0 \\ 0 & -\frac{D+\nu_3}{Y_S} & -\lambda & \nu_3 \\ -\frac{\nu_1 F}{K_F+F} & \frac{\nu_1 F}{K_F+F} & 0 & -D-\lambda \end{vmatrix} = 0 \tag{24}$$

It can be shown that all eigenvalues have negative real parts if the equilibrium concentrations are all positive, and that this equilibrium is therefore stable under those conditions. Now let us introduce a second species, which competes for *S* as limiting substrate. The set of differential equations becomes

$$\frac{\partial F}{\partial t} = D(F_{in} - F) - \frac{\nu_1 E F}{K_F + F}\ , \tag{25a}$$

$$\frac{\partial S}{\partial t} = \frac{\nu_1 E F}{K_F + F} - \frac{V_{2,1} X_1 S}{K_{S,1} + S} - \frac{V_{2,2} X_2 S}{K_{S,2} + S} - DS\,, \tag{25b}$$

$$\frac{\partial X_1}{\partial t} = Y_{S,1} \frac{V_{2,1} X_1 S}{K_{S,1} + S} - DX_1 - \nu_3 X_1\,, \tag{25c}$$

$$\frac{\partial X_2}{\partial t} = Y_{S,2}\frac{V_{2,2}X_2 S}{K_{S,2}+S} - DX_2\,, \tag{25d}$$

$$\frac{\partial E}{\partial t} = -DE + v_3 X_1\ . \tag{25e}$$

It can readily be shown that the (25c) and (25d) yield conflicting equilibrium substrate concentrations (except for very rare cases):

$$S_{eq,1} = \frac{D+v_3}{Y_{S,1}V_{2,1} - D - v_3}K_{S,1} \approx \frac{D}{a^0_{S,1}}, \tag{26a}$$

$$S_{eq,2} = \frac{D}{Y_{S,2}V_{2,2} - D}K_{S,2} \approx \frac{D}{a^0_{S,2}}. \tag{26b}$$

The approximation holds for dilution rates well below the maximum specific growth rate. It can now be shown that there is no two-species equilibrium in this case, and that transient introduction of a high specific affinity competitor may destabilize the single species equilibrium of fibrolytic bacteria, even leading to their extinction. If low specific affinity species cannot compete with the fibrolytic species. This explains why fibrolysis takes time: only high specific affinity, low maximum growth rate bacteria can afford a fibrolytic lifestyle.

The situation is different if the substrate *S* is used by other bacteria, but not as limiting substrate. In that case the competing species are held at a constant level (e.g., dependent on some other substrate), and only equation (22b) need be adapted to

$$\frac{\partial S}{\partial t} = \frac{v_1 EF}{K_F + F} - \frac{V_{2,1}X_1 S}{K_{S,1}+S} - \left(D + V_{2,2}X_2\right)S. \tag{27}$$

The equilibrium is similar

$$E_{eq} = \frac{v_3 X_{eq}}{D} \tag{28a}$$

$$S_{eq} = \frac{D+v_3}{Y_s v_2 - D - v_3}K_S \tag{28b}$$

$$F_{eq} = \frac{F_{in} - K_F - \frac{v_1 v_3 Y_S}{D(D+v_3)} \pm \sqrt{\left(F_{in} - K_F - \frac{v_1 v_3 Y_S}{D(D+v_3)}\right)^2 + 4F_{in}K_F\left(1 - \frac{v_1 v_3 Y_S\left(F_{in} - \left(D+V_{2,2}X_2\right)S_{eq}/D\right)}{D(D+v_3)}\right)}}{2\left(1 - \frac{v_1 v_3 Y_S\left(F_{in} - \left(D+V_{2,2}X_2\right)S_{eq}/D\right)}{D(D+v_3)}\right)} \quad (28c)$$

$$X_{1,eq} = Y_s \frac{F_{in} - F_{eq} - \left(D+V_{2,2}X_2\right)S_{eq}/D}{1+v_3/D}. \quad (28d)$$

The equilibrium is again stable, though a higher input fibre concentration is needed to ensure survival. This case is important because it is identical in behaviour to "parasitic" competition by the host.

3.3 Toxin inactivation

Toxins of any kind (cidal or inhibitory) may be inactivated in two distinct ways: (i) by uptake of the toxin (e.g., used as metabolite), and (ii) by secretion of enzymes or other inactivating agents. The first case was modelled in the MIMICS pilot study [Wilkinson, 1997], in the interaction between anaerobes and aerobes. In this case we have non-competitive inhibition and/or cidal effect of oxygen (O) of anaerobes, and uptake of oxygen by aerobes. In the case of inhibition the differential equations are

$$\frac{\partial S}{\partial t} = D(S_{in} - S) - V_{max,1}\frac{S}{K_{S,1}+S}\frac{K_{inh,1}}{K_{inh,1}+O}X_1 - V_{max,2}\frac{S}{K_{S,2}+S}\frac{O}{K_O+O}X_2, \quad (29a)$$

$$\frac{\partial X_1}{\partial t} = Y_S V_{max,1}\frac{S}{K_{S,1}+S}\frac{K_{inh,1}}{K_{inh,1}+O}X_1 - DX_1. \quad (29b)$$

$$\frac{\partial X_2}{\partial t} = Y_S V_{max,2}\frac{S}{K_{S,2}+S}\frac{O}{K_O+O}X_2 - DX_2. \quad (29c)$$

$$\frac{\partial O}{\partial t} = D(O_{in} - O) - V_{max,1}\frac{S}{K_{S,1}+S}\frac{O}{K_{inh,1}+O}X_1 - V_{max,O}\frac{S}{K_{S,2}+S}\frac{O}{K_O+O}X_2. \quad (29d)$$

In the purely cidal case we have

$$\frac{\partial S}{\partial t} = D(S_{in} - S) - V_{max,1}\frac{S}{K_{S,1}+S}X_1 - V_{max,2}\frac{S}{K_{S,2}+S}\frac{O}{K_O+O}X_2, \quad (30a)$$

$$\frac{\partial X_1}{\partial t} = Y_S V_{max,1} \frac{S}{K_{S,1} + S} X_1 - \left(D + \kappa_{tox} O\right) X_1 . \tag{30b}$$

$$\frac{\partial X_2}{\partial t} = Y_S V_{max,2} \frac{S}{K_{S,2} + S} \frac{O}{K_O + O} X_2 - D X_2 . \tag{30c}$$

$$\frac{\partial O}{\partial t} = D\left(O_{in} - O\right) - \beta_{tox} X_1 O - V_{max,O} \frac{S}{K_{S,2} + S} \frac{O}{K_O + O} X_2 . \tag{30d}$$

If inactivating enzymes (*E*) directed against an inhibitory toxin *T* are produced at a rate v_E, we have

$$\frac{\partial S}{\partial t} = D\left(S_{in} - S\right) - V_{max,1} \frac{S}{K_{S,1} + S} \frac{K_{inh,1}}{K_{inh,1} + T} X_1 - V_{max,2} \frac{S}{K_{S,2} + S} X_2 , \tag{31a}$$

$$\frac{\partial X_1}{\partial t} = Y_S V_{max,1} \frac{S}{K_{S,1} + S} \frac{K_{inh,1}}{K_{inh,1} + T} X_1 - D X_1 . \tag{31b}$$

$$\frac{\partial X_2}{\partial t} = Y_S V_{max,2} \frac{S}{K_{S,2} + S} X_2 - \left(D - v_E\right) X_2 . \tag{31c}$$

$$\frac{\partial E}{\partial t} = v_E X_2 - D E . \tag{31d}$$

$$\frac{\partial T}{\partial t} = D\left(T_{in} - T\right) - V_{max,1} \frac{S}{K_{S,1} + S} \frac{T}{K_{inh,1} + T} X_1 - V_{max,T} \frac{ET}{K_T + T} . \tag{31e}$$

For a cidal toxin we have

$$\frac{\partial S}{\partial t} = D\left(S_{in} - S\right) - V_{max,1} \frac{S}{K_{S,1} + S} X_1 - V_{max,2} \frac{S}{K_{S,2} + S} X_2 , \tag{32a}$$

$$\frac{\partial X_1}{\partial t} = Y_S V_{max,1} \frac{S}{K_{S,1} + S} X_1 - \left(D + \kappa_{tox} T\right) X_1 . \tag{32b}$$

$$\frac{\partial X_2}{\partial t} = Y_S V_{max,2} \frac{S}{K_{S,2} + S} X_2 - \left(D - v_E\right) X_2 . \tag{32c}$$

$$\frac{\partial E}{\partial t} = v_E X_2 - D E . \tag{32d}$$

$$\frac{\partial T}{\partial t} = D(T_{in} - T) - \beta_{tox} X_1 T - V_{max,T} \frac{ET}{K_T + T}. \tag{32e}$$

The earlier, computer simulation work in the project has provided insight into this type of interaction within a certain range of parameters [Wilkinson, 1997]. This showed that situations may arise in which the toxin inactivating strain and toxin sensitive strains may coexist, and that the trivial equilibrium without any bacteria is only unstable for invasion by inactivators. Once these are present, the sensitive strains may arise. Both single species equilibria can be stable, except for invasion by the other species. Only the two-species equilibrium is truly stable, which is the reverse situation of that discussed in section 3.5 on toxin production. Further analysis of the behaviour of the pure forms of this type of interaction will be done in future work, however, the next section describes a combination of this type of effect with another interaction, viz. production of substrate by the sensitive species.

3.4 Symbiotic food interactions.

Here we model toxic metabolites and mutualism through use of those metabolites by other bacteria. Suppose we have two species, X_1 living off substrate S_1 and producing metabolite S_2, and X_2 living off S_2. Maximum specific uptake rates and Michaelis-Menten constants are v_1, v_2, K_1 and K_2, respectively. The yield of biomass per unit of substrate is Y_s for both species, and the yield of metabolite S_2 by X_1 per unit of substrate used is $Y_{m,1}$. Furthermore, we assume that growth of X_1 is inhibited (non-competitively) by its metabolite S_2, with an inhibition constant of K_T. We then arrive at the following set of differential equations:

$$\frac{\partial S_1}{\partial t} = D(S_{in} - S_1) - \frac{v_1 X_1 S_1}{K_1 + S_1} \frac{K_T}{K_T + S_2} \tag{33a}$$

$$\frac{\partial S_2}{\partial t} = Y_{m,1} \frac{v_1 X_1 S_1}{K_1 + S_1} \frac{K_T}{K_T + S_2} - \frac{v_2 X_2 S_2}{K_2 + S_2} - D S_2 \tag{33b}$$

$$\frac{\partial X_1}{\partial t} = Y_s \frac{v_1 X_1 S_1}{K_1 + S_1} \frac{K_T}{K_T + S_2} - D X_1 \tag{33c}$$

$$\frac{\partial X_2}{\partial t} = Y_s \frac{v_2 X_2 S_2}{K_2 + S_2} - D X_2 \tag{33d}$$

Obviously, the second species can only survive if the first is present. The two-species equilibrium is at:

$$S_2 = \frac{D K_2}{Y_s v_2 - D} \tag{34a}$$

$$S_1 = \frac{D K_1}{Y_s v_1 K_T / (K_T + S_2) - D} \tag{34b}$$

$$X_1 = Y_s (S_{in} - S_1) \tag{34c}$$

$$X_2 = Y_{m,1} X_1 - Y_s S_2 = Y_s \left(Y_{m,1} (S_{in} - S_1) - S_2 \right) \tag{34d}$$

The Jacobian is:

$$\mathbf{J} = \begin{pmatrix} -D - \frac{v_1 X_1 K_1}{(K_1 + S_1)^2} \frac{K_T}{K_T + S_2} & Y_{m,1} \frac{v_1 X_1 K_1}{(K_1 + S_1)^2} \frac{K_T}{K_T + S_2} & Y_s \frac{v_1 X_1 K_1}{(K_1 + S_1)^2} \frac{K_T}{K_T + S_2} & 0 \\ \frac{v_1 X_1 S_1}{K_1 + S_1} \frac{K_T}{(K_T + S_2)^2} & -Y_{m,1} \frac{v_1 X_1 S_1}{K_1 + S_1} \frac{K_T}{(K_T + S_2)^2} - \frac{v_2 X_2 K_2}{(K_2 + S_2)^2} - D & -Y_s \frac{v_1 X_1 S_1}{K_1 + S_1} \frac{K_T}{(K_T + S_2)^2} & Y_s \frac{v_2 X_2 K_2}{(K_2 + S_2)^2} \\ -\frac{v_1 S_1}{K_1 + S_1} \frac{K_T}{K_T + S_2} & Y_{m,1} \frac{v_1 S_1}{K_1 + S_1} \frac{K_T}{K_T + S_2} & Y_s \frac{v_1 S_1}{K_1 + S_1} \frac{K_T}{K_T + S_2} - D & 0 \\ 0 & -\frac{v_2 S_2}{K_2 + S_2} & 0 & Y_s \frac{v_2 K_2}{K_2 + S_2} - D \end{pmatrix}$$

at the point of equilibrium the eigenvalue equation becomes:

$$\begin{vmatrix} -D - \frac{v_1 X_1 K_1}{K_1 + S_1} \frac{D}{Y_s S_1} - \lambda & Y_{m,1} \frac{v_1 X_1 K_1}{K_1 + S_1} \frac{D}{Y_s S_1} & \frac{v_1 X_1 K_1}{K_1 + S_1} \frac{D}{S_1} & 0 \\ \frac{D X_1}{Y_s (K_T + S_2)} & -Y_{m,1} \frac{D X_1}{Y_s (K_T + S_2)} - \frac{v_2 X_2 K_2}{(K_2 + S_2)^2} - D - \lambda & -\frac{D X_1}{(K_T + S_2)} & Y_s \frac{v_2 X_2 K_2}{(K_2 + S_2)^2} \\ -\frac{D}{Y_s} & Y_{m,1} \frac{D}{Y_s} & -\lambda & 0 \\ 0 & -\frac{D}{Y_s} & 0 & -\lambda \end{vmatrix} = 0 \tag{35}$$

It can be shown that all coefficients in the fourth order equation above are larger than zero, provided all equilibrium concentrations are positive, and that therefore all real components of the roots of this equation are negative. The equilibrium is therefore stable.

3.5 Toxin production:

3.5.1 The action of bacteriocins

Frank [1994] has produced a theoretical model of competition through bacteriocins. He found that two stable equilibria may be present, unlike in the case of food competition. However, his treatment uses logistic growth as a model for bacterial growth, which is not satisfactory [Koch, 1997].

Consider a system of two species, one susceptible X_{susc}, one resistant X_{prod} and producing a toxin *T*, competing for a single substrate *S*. Both species have the same uptake parameters μ_{max}, V_{max} and K_S. As in the case of Frank [1994], we assume that X_{prod} produces *T* at a constant rate a_{tox} per unit of biomass (basal metabolism), and *T* is cidal rather than inhibitory. The yield is set at 1 without loss of generality. The toxin kills susceptibles at a rate κ_{tox} per unit of biomass per unit of toxin. Toxin is removed from the system by this reaction at a rate β_{tox} per unit of biomass per unit of toxin. Lysis of killed cells returns a fraction Y_κ of the biomass as substrate. Using Monod formulation explicitly in a chemostat at dilution rate *D*, we find that the appropriate set of differential equations is:

$$\frac{\partial X_{prod}}{\partial t} = \mu_{max}\frac{S}{K_S+S}X_{prod} - (D + a_{tox})X_{prod} \tag{36a}$$

$$\frac{\partial T}{\partial t} = a_{tox}X_{prod} - (D + \beta_{tox}X_{susc})T \tag{36b}$$

$$\frac{\partial X_{susc}}{\partial t} = \mu_{max}\frac{S}{K_S+S}X_{susc} - (D + \kappa_{tox}T)X_{susc} \tag{36c}$$

$$\frac{\partial S}{\partial t} = D(S_{in} - S) - V_{max}\frac{S}{K_S+S}(X_{susc} + X_{prod}) + Y_\kappa\kappa_{tox}TX_{susc} \tag{36d}$$

As in the logistic case described by Frank, three non-trivial equilibria exist. One has only susceptibles:

$$X_{prod} = 0 \tag{37a}$$

$$S = \frac{D}{\mu_{max} - D}K_S \tag{37b}$$

$$X_{susc} = \frac{\mu_{max}}{V_{max}}\left(S_{in} - \frac{D}{\mu_{max} - D}K_S\right) \tag{37c}$$

$$T = 0 \tag{37d}$$

Stability analysis shows that this solution is positive if the concentrations are all non-negative in steady state, and hence $\mu_{max} > D$ and $S_{in} \geq \frac{D}{\mu_{max} - D}K_S$.

The next equilibrium has only producers:

$$X_{susc} = 0 \tag{38a}$$

$$S = \frac{D + a_{tox}}{\mu_{max} - (D + a_{tox})}K_S \tag{38b}$$

$$X_{prod} = \frac{\mu_{max}}{V_{max}}\left(\frac{D}{D + a_{tox}}S_{in} - \frac{D}{\mu_{max} - (D + a_{tox})}K_S\right) \tag{38c}$$

$$T = \frac{a_{tox}}{D}X_{prod} = \frac{\mu_{max}}{V_{max}}\left(\frac{a_{tox}}{D + a_{tox}}S_{in} - \frac{a_{tox}}{\mu_{max} - (D + a_{tox})}K_S\right) \tag{38d}$$

The stability requirement becomes:

$$\lambda^4 + \left(V_{max}\frac{K_S X_{prod}}{(K_S + S)^2} + 2D + \kappa_{tox}T - a_{tox}\right)\lambda^3 +$$
$$\left(V_{max}\frac{K_S X_{prod}}{(K_S + S)^2}(2D + a_{tox}) + \left(V_{max}\frac{K_S X_{prod}}{(K_S + S)^2} + 2D\right)(\kappa_{tox}T - a_{tox}) + D^2\right)\lambda^2 +$$
$$\left(\left(V_{max}D\frac{K_S X_{prod}}{(K_S + S)^2} + D^2\right)(\kappa_{tox}T - a_{tox}) + V_{max}\frac{K_S X_{prod}}{(K_S + S)^2}(2D + a_{tox})(D + \kappa_{tox}T - a_{tox})\right)\lambda +$$
$$V_{max}\frac{K_S X_{prod}}{(K_S + S)^2}(2D^2 + Da_{tox})(\kappa_{tox}T - a_{tox}) = 0$$

As in the above case, the system is stable only if the concentrations of the steady solution are positive. In addition, only if

$$\kappa_{tox} T > a_{tox} \qquad \Leftrightarrow \qquad \kappa_{tox} \frac{X_{prod}}{D} > 1 \tag{39}$$

are all coefficients positive, and are all real parts of the roots negative. Therefore, only if a sufficient number of producers are present in steady state can they outcompete the susceptibles. Since we can rewrite the condition as

$$\frac{\kappa_{tox} \mu_{max}}{V_{max}} \left(\frac{S_{in}}{D + a_{tox}} - \frac{K_S}{\mu_{max} - (D + a_{tox})} \right) > 1, \tag{40}$$

we see that this equilibrium is more stable as the input substrate concentration increases. Finally we have coexistence of the two species:

$$T = \frac{a_{tox}}{\kappa_{tox}} \tag{41a}$$

$$S = \frac{D + a_{tox}}{\mu_{max} - (D + a_{tox})} K_S \tag{41b}$$

$$X_{susc} = \frac{Y_S \kappa_{tox} \left(\frac{D}{D + a_{tox}} S_{in} - \frac{DK_S}{\mu_{max} - (D + a_{tox})} \right) - D}{\kappa_{tox} + \beta_{tox} - \kappa_{tox} \frac{\mu_{max}}{V_{max}} \frac{Y_\kappa a_{tox}}{D + a_{tox}}} \tag{41c}$$

$$X_{prod} = \frac{Y_S \beta_{tox} \left(\frac{D}{D + a_{tox}} S_{in} - \frac{DK_S}{\mu_{max} - (D + a_{tox})} \right) + \left(1 - \frac{\mu_{max}}{V_{max}} \frac{Y_\kappa a_{tox}}{D + a_{tox}} \right) D}{\kappa_{tox} + \beta_{tox} - \kappa_{tox} \frac{\mu_{max}}{V_{max}} \frac{Y_\kappa a_{tox}}{D + a_{tox}}} \tag{41d}$$

This equilibrium cannot be stable if the two single species equilibria are stable (stable and unstable equilibria must alternate).

However, if we have an inhibitory bacteriocin the equations become (using non-competitive inhibition), the differential equations become

$$\frac{\partial X_{prod}}{\partial t} = \mu_{max} \frac{S}{K_S + S} X_{prod} - (D + a_{tox}) X_{prod} \tag{42a}$$

$$\frac{\partial T}{\partial t} = a_{tox} X_{prod} - DT \tag{42b}$$

$$\frac{\partial X_{susc}}{\partial t} = \mu_{max} \frac{S}{K_S + S} \frac{K_{tox}}{K_{tox} + T^n} X_{susc} - DX_{susc} \tag{42c}$$

$$\frac{\partial S}{\partial t} = D(S_{in} - S) - V_{max} \frac{S}{K_S + S} \left(\frac{K_{tox}}{K_{tox} + T^n} X_{susc} + X_{prod} \right) \tag{42d}$$

The two single species equilibria are identical to the case above, but the two-species equilibrium becomes:

$$S = \frac{D + a_{tox}}{\mu_{max} - (D + a_{tox})} K_S \tag{43a}$$

$$T = \sqrt[n]{\frac{K_{tox} a_{tox}}{D}} \tag{43b}$$

$$X_{prod} = \frac{D}{a_{tox}} \sqrt[n]{\frac{K_{tox} a_{tox}}{D}} \tag{43c}$$

$$X_{susc} = Y_S (S_{in} - S) - \frac{D + a_{tox}}{a_{tox}} \sqrt[n]{\frac{K_{tox} a_{tox}}{D}} \tag{43d}$$

In the case of $n = 1$ we have:

$$T = \frac{K_{tox} a_{tox}}{D} \tag{44a}$$

$$X_{prod} = \frac{K_{tox}}{a_{tox}} \tag{44b}$$

$$X_{susc} = Y_S (S_{in} - S) - \frac{D + a_{tox}}{D} K_{tox} \tag{44c}$$

The stability requirement for the equilibrium with only producers (for n=1) reduces to

$$a_{tox} < \frac{DT}{K_{tox}} = \frac{a_{tox}}{K_{tox}} X_{prod} \qquad \Leftrightarrow \qquad \frac{X_{prod}}{K_{tox}} > 1 \tag{45a}$$

or

$$\frac{\mu_{max}}{K_{tox}V_{max}}\left(\frac{D}{D+a_{tox}}S_{in}-\frac{D}{\mu_{max}-(D+a_{tox})}K_S\right)>1 \tag{45b}$$

The steady state behaviour is therefore similar to the logistic case discussed by Frank. However, dynamic behaviour of Monod and logistic models can be very different [Wilkinson, submitted].

3.5.2 Toxic by-products of substrate uptake

A modification is needed to model toxic substances produced in the course of uptake. In this case the set of differential equations becomes

$$\frac{\partial X_{prod}}{\partial t}=(\mu_{max}-a_{tox})\frac{S}{K_S+S}X_{prod}-DX_{prod} \tag{46a}$$

$$\frac{\partial T}{\partial t}=a_{tox}\frac{S}{K_S+S}X_{prod}-(D+\beta_{tox}X_{susc})T \tag{46b}$$

$$\frac{\partial X_{susc}}{\partial t}=\mu_{max}\frac{S}{K_S+S}X_{susc}-(D+\kappa_{tox}T)X_{susc} \tag{46c}$$

$$\frac{\partial S}{\partial t}=D(S_{in}-S)-V_{max}\frac{S}{K_S+S}(X_{susc}+X_{prod})+Y_{\kappa}\kappa_{tox}TX_{susc} \tag{46d}$$

The equilibrium of only susceptibles is identical to that in the previous case. However, the equilibrium of producers becomes

$$X_{susc}=0 \tag{47a}$$

$$S=\frac{D}{\mu_{max}-a_{tox}-D}K_S \tag{47b}$$

$$X_{prod}=\frac{\mu_{max}-a_{tox}}{V_{max}}\left(S_{in}-\frac{D}{\mu_{max}-a_{tox}-D}K_S\right) \tag{47c}$$

$$T=\frac{a_{tox}}{\mu_{max}-a_{tox}}X_{prod}=\frac{a_{tox}}{V_{max}}\left(S_{in}-\frac{D}{\mu_{max}-a_{tox}-D}K_S\right) \tag{47d}$$

The stability requirement becomes:

$$\begin{vmatrix} -\lambda & 0 & a_{tox} & -V_{max}\dfrac{D}{\mu_{max}-a_{tox}} \\ 0 & \dfrac{a_{tox}D}{\mu_{max}-a_{tox}}-\kappa_{tox}T-\lambda & -\beta_{tox}T & -V_{max}\dfrac{D}{\mu_{max}-a_{tox}}+Y_{\kappa}\kappa_{tox}T \\ 0 & 0 & -D-\lambda & 0 \\ (\mu_{max}-a_{tox})\dfrac{K_S X_{prod}}{(K_S+S)^2} & 0 & 0 & -V_{max}\dfrac{K_S X_{prod}}{(K_S+S)^2}-D-\lambda \end{vmatrix} = 0$$

As in the above case, the system is stable only if the concentrations of the steady solution are positive. In addition, only if

$$\kappa_{tox}T > \frac{a_{tox}D}{\mu_{max}-a_{tox}} \qquad \Leftrightarrow \qquad \kappa_{tox}\frac{X_{prod}}{D} > 1 \tag{48}$$

This criterion is identical to the case of constant toxin production. However, if we fill in the equilibrium value for X_{prod}, we have

$$\kappa_{tox}\frac{\mu_{max}-a_{tox}}{V_{max}}\left(\frac{S_{input}}{D}-\frac{K_S}{\mu_{max}-a_{tox}-D}\right) > 1\,. \tag{49}$$

Though slightly different from the previous case, the overall behaviour is quite similar. The effect of shunting down of the toxin production due to decreased substrate levels at equilibrium is twofold: (i) toxic action towards competitors is reduced, and (ii) biomass losses of the producer are reduced. These two effects clearly cancel out almost completely. As long as a_{tox} is small compared to μ_{max}, it is κ_{tox} that determines the success of toxin production strategies.

3.6 Predator-prey interactions

The prey species can be modelled using Monod formalism, the predator by Holling type II (equivalent to Monod), or other scheme with predator satiation. Lotka-Volterra type oscillations may occur. The set differential equation becomes

$$\frac{\partial X_1}{\partial t} = \mu_{max,1}\frac{S}{K_{S,1}+S}X_1 - V_{max,2}\frac{X_1}{K_X+X_1}X_2 - DX_1 \tag{50a}$$

$$\frac{\partial X_2}{\partial t} = \mu_{max,2} \frac{X_1}{K_X + X_1} X_2 - DX_2 \tag{50b}$$

$$\frac{\partial S}{\partial t} = D(S_{in} - S) - V_{max,1} \frac{S}{K_{S,1} + S} X_1 \tag{50c}$$

The equilibrium is at

$$X_1 = \frac{DK_X}{\mu_{max,2} - D} \tag{51a}$$

$$S = \frac{1}{2}\left(S_{in} - K_{S,1} - \frac{V_{max,1}K_X}{\mu_{max,2} - D} \pm \sqrt{\left(S_{in} - K_{S,1} - \frac{V_{max,1}K_X}{\mu_{max,2} - D} \right)^2 + 4K_{S,1}S_{in}} \right) \tag{51b}$$

$$X_2 = \frac{\mu_{max,2}}{V_{max,2}} \left(\frac{\mu_{max,1}}{V_{max,1}} (S_{in} - S) - X_1 \right) \tag{51c}$$

The Jacobian is given by

$$\mathbf{J} = \begin{pmatrix} \frac{\mu_{max,1}S}{K_{S,1}+S} - \frac{V_{max,2}K_X}{(K_X+X_1)^2}X_2 - D & \frac{\mu_{max,2}K_X}{(K_X+X_1)^2}X_2 & -\frac{V_{max,1}S}{K_{S,1}+S} \\ -\frac{V_{max,2}X_1}{K_X+X_1} & \frac{\mu_{max,2}X_1}{K_X+X_1} - D & 0 \\ \frac{\mu_{max,1}K_{S,1}X_1}{(K_{S,1}+S)^2} & 0 & -D - \frac{V_{max,1}K_{S,1}X_1}{(K_{S,1}+S)^2} \end{pmatrix}, \tag{52}$$

and the eigenvalue equation is

$$\begin{vmatrix} \frac{V_{max,2}DX_2}{\mu_{max,2}(K_X+X_1)} - \lambda & \frac{\mu_{max,2}K_X}{(K_X+X_1)^2}X_2 & -\frac{V_{max,1}S}{K_{S,1}+S} \\ -\frac{V_{max,2}D}{\mu_{max,2}} & -\lambda & 0 \\ \frac{\mu_{max,1}K_{S,1}X_1}{(K_{S,1}+S)^2} & 0 & -D - \frac{V_{max,1}K_{S,1}X_1}{(K_{S,1}+S)^2} - \lambda \end{vmatrix} = 0 \tag{53}$$

It can be shown that this equilibrium can be stable, unstable, or metastable, depending in principle on the food supply, and especially the ratio of the input substrate concentrations to

the Michaelis-Menten constants. As the ecosystem is enriched, it becomes more and more unstable [DeAngelis, 1992]. Apart from predatory bacteria such as *Bdellovibrio bacteriovorus*, these equations also hold for bacterium-phage systems.

3.7 Decoys in predator-prey interactions

A simple reasoning can show that species which cannot themselves act as prey, and do not compete with the prey, can interfere in a predator-prey system [Wilkinson, submitted]. We will now take the case of a three-species ecosystem: one prey species X_1, one non-prey species X_2, and a predator *Y*. Disregarding starvation, the predator can be in three states: free, bound to X_1, and bound to X_2. The assume the rate of collisions is *r* per unit of prey or non-prey species per unit of predator. Since chemotaxis towards prey has not been observed in *Bdellovibrio* spp. (8), this rate is assumed to be identical for prey and non-prey species. Furthermore, the prey/predator complex dissociates at a rate of k_1, and non-prey/predator complex dissociates at a rate of k_2. However, only the dissociation of the first complex yields new predators, with a yield of y_x.

The central assumption of this theory is that after colliding with a non-prey cell, a predator will briefly attach, before detaching. Such behaviour has been observed in the *Neisseria gonorrhoeae - B. bacteriovorus* system [Drutz, 1976]. The rate constant k_2 will therefore be finite. This leads to the following set of differential equations:

$$\frac{\partial [Y_{free}]}{\partial t} = (y_x + 1)k_1[X_1Y] + k_2[X_2Y] - r([X_1] + [X_2])[Y_{free}] \tag{54a}$$

$$\frac{\partial [X_1Y]}{\partial t} = -k_1[X_1Y] + r[X_1][Y_{free}] \tag{54b}$$

$$\frac{\partial [X_2Y]}{\partial t} = -k_2[X_2Y] + r[X_2][Y_{free}]. \tag{54c}$$

At steady state we have

$$[X_1Y] = \frac{r}{k_1}[X_1][Y_{free}] \quad \text{and} \quad [X_2Y] = \frac{r}{k_2}[X_2][Y_{free}].$$

Remembering that the total predator content $[Y]$ is

$$[Y] = [X_1 Y] + [X_2 Y] + [Y_{free}] = \left(1 + \frac{r}{k_1}[X_1] + \frac{r}{k_2}[X_2]\right)[Y_{free}]$$

and summing the equations (54a,b,c) we find a growth rate of:

$$\frac{\partial [Y]}{\partial t} = \frac{y_x k_1 [X_1][Y]}{k_1/r + [X_1] + k_1[X_2]/k_2} = \frac{\mu_x [X_1][Y]}{K_x + [X_1] + K_{inh}[X_2]}, \tag{55}$$

in which we recognize the standard form of competitive inhibition. Therefore, in an extremely densely populated *and* diverse ecosystem, such as the intestinal microflora, specialist predators would be in a serious disadvantage compared to generalists. We can absorb the "decoy effect" into the Michaelis-Menten constant of the predator setting

$$K_X^* = K_X + K_{inh}[X_2]. \tag{56}$$

This shows that the effective Michaelis-Menten constant increases, and that therefore the ecosystem should become more stable (see previous section). Since instability is needed in therapeutic use of phages or predatory bacteria, this effect may explain the frequent failure of such schemes. The reasoning (and differential equations) used above can be used with little or no adaptation for lytic bacteriophages, except they do not starve in the absence of food.

3.8 Direct binding site competition

Suppose bacteria can be in two states: (i) X_b bound to the wall, and (ii) X_f free in the lumen. Furthermore, suppose that *N* species are competing for a maximum of $X_{b,max}$ binding sites. Furthermore, suppose that a cell of species *i* bound to the wall has a Michaelis-Menten constant $K_{b,i}$, and maximum specific growth rate $\mu_{b,i}$. Similarly, for free cells we have a Michaelis-Menten constant $K_{f,i}$, and maximum specific growth rate $\mu_{f,i}$. Furthermore, we assume that the probability of daughter cell of a bound cell binding to wall immediately *if there is a place available* is *p*. The probability that a place is available is

proportional to the number of free sites. The rest of the daughters of bound cells are shed into the lumen. If the rate of attachment of free cells is $r_{b,1}$, the rate of sloughing of bound cells is $D_{sl,i}$, and the dilution rate of the lumen is *D*, we arrive at the following set of differential equations:

$$\begin{aligned}\frac{\partial X_{b,i}}{\partial t} &= p\left(1-\frac{1}{X_{b,max}}\sum_{i=1}^{N}X_{b,i}\right)\mu_{b,i}\frac{S}{K_{b,i}+S}X_{b,i} \\ &\quad + r_{b,1}\left(1-\frac{1}{X_{b,max}}\sum_{i=1}^{N}X_{b,i}\right)X_{f,i} - D_{sl,i}X_{b,i}\end{aligned} \tag{57a}$$

$$\begin{aligned}\frac{\partial X_{f,i}}{\partial t} &= \left(1-p\left(1-\frac{1}{X_{b,max}}\sum_{i=1}^{N}X_{b,i}\right)\right)\frac{\mu_{b,i}S}{K_{b,i}+S}X_{b,i} + \frac{\mu_{f,i}S}{K_{f,i}+S}X_{f,i} \\ &\quad - r_{b,i}\left(1-\frac{1}{X_{b,max}}\sum_{i=1}^{N}X_{b,i}\right)X_{f,i} - DX_{f,i} + D_{sl,i}X_{b,i}\end{aligned} \tag{57b}$$

$$\frac{\partial S}{\partial t} = D\left(S_{in}-S\right) - \sum_{i=1}^{N}V_{b,i}\frac{S}{K_{f,i}+S}X_{b,i} + V_{f,i}\frac{S}{K_{f,i}+S}X_{f,i}\,. \tag{57c}$$

Even in the simple case of *N*=2, and using a number of simplifications, it has not been possible to find analytical solutions to date. Further work is needed on this point.

3.9 Indirect binding site competition

The above interaction becomes even more complicated when bacteria can reduce the probability of attachment of other species. The above set of differential equations must then be modified, by allowing species X_1 to secrete a substance *T*, which influences the probability of attachment of other species. We assume that the secreted substance is autoinhibitory, i.e., its production is inhibited by its presence. This prevents the concentration of *T* becoming arbitrarily high.

Differential equations for this situation must still be developed.

3.10 Biofilm gel production

A further adaptation of the mucosal model divides each species of bacteria into three compartments: lumen, free within biofilm gel, and bound to the wall. If we denote the

luminal fraction of species X_i and substrate S as $X_{l,i}$ and S_l, respectively, and retain the notation in (57) for free living in the biofilm and bound bacteria, we have:

$$\begin{aligned}\frac{\partial X_{b,i}}{\partial t} &= p\left(1-\frac{1}{X_{b,max}}\sum_{i=1}^{N}X_{b,i}\right)\mu_{b,i}\frac{S}{K_{b,i}+S}X_{b,i} \\ &\quad +r_{b,1}\left(1-\frac{1}{X_{b,max}}\sum_{i=1}^{N}X_{b,i}\right)X_{f,i}-D_{sl,i}X_{b,i}\end{aligned} \tag{58a}$$

$$\begin{aligned}\frac{\partial X_{f,i}}{\partial t} &= \left(1-p\left(1-\frac{1}{X_{b,max}}\sum_{i=1}^{N}X_{b,i}\right)\right)\frac{\mu_{b,i}S}{K_{b,i}+S}X_{b,i}+\frac{\mu_{f,i}S}{K_{f,i}+S}X_{f,i} \\ &\quad -r_{b,i}\left(1-\frac{1}{X_{b,max}}\sum_{i=1}^{N}X_{b,i}\right)X_{f,i}-D_{diff,i}\left(X_{f,i}-X_{l,i}\right)+D_{sl,i}X_{b,i}\end{aligned}, \tag{58b}$$

$$\frac{\partial X_{l,i}}{\partial t} = \frac{\mu_{l,i}S_l}{K_{l,i}+S_l}X_{l,i}-D_{diff,i}\left(X_{l,i}-X_{f,i}\right)-DX_{l,i}, \tag{58c}$$

$$\frac{\partial S}{\partial t} = D_{diff,S}\left(S_l-S\right)-\sum_{i=1}^{N}V_{b,i}\frac{S}{K_{f,i}+S}X_{b,i}+V_{f,i}\frac{S}{K_{f,i}+S}X_{f,i}, \tag{58d}$$

$$\frac{\partial S_l}{\partial t} = D\left(S_{l,in}-S_l\right)-\sum_{i=1}^{N}V_{l,i}\frac{S_l}{K_{l,i}+S_l}X_{l,i}. \tag{58e}$$

In this model the diffusion constants $D_{diff,i}$ determine the rate of exchange of material from biofilm gel to the lumen. This diffusion constant can be made a function of biofilm composition. By doing so, bacteria may influence this parameter by secreting polymers which decrease diffusion.

Differential equations for this type of interaction have not yet been drawn up, analysis of the steady state cannot yet be performed.

3.11 Biofilm gel destruction

Same case as above, except that bacteria may also degrade the polymers which decrease the diffusion rate. This allows them to detach when conditions are adverse. Equations describing this type of behaviour will be drawn up in the future.

3.12 "Meta-adherence"

Bacteria may adhere to each other, creating very complex communities. Again, a species which adheres to a wall bound bacterium may be modelled by splitting the species into several compartments in the model. In the two species case we have

$$\frac{\partial X_{b,1}}{\partial t} = p\left(1-\frac{X_{b,1}}{X_{b,max}}\right)\frac{\mu_{b,1}S}{K_{b,1}+S}X_{b,1} + r_{b,1}\left(1-\frac{X_{b,1}}{X_{b,max}}\right)X_{f,1} - D_{sl,1}X_{b,1} \tag{59a}$$

$$\frac{\partial X_{b,2}}{\partial t} = p\left(1-\frac{X_{b,2}}{\varepsilon X_{b,1}}\right)\frac{\mu_{b,2}S}{K_{b,2}+S}X_{b,2} + r_{b,2}\left(1-\frac{X_{b,2}}{\varepsilon X_{b,1}}\right)X_{f,2} - \left(D_{sl,1}+D_{sl,2}\right)X_{b,2} \tag{59b}$$

$$\begin{aligned}\frac{\partial X_{f,1}}{\partial t} = &\left(1-p\left(1-\frac{X_{b,1}}{X_{b,max}}\right)\right)\frac{\mu_{b,1}S}{K_{b,1}+S}X_{b,1} + \frac{\mu_{f,1}S}{K_{f,1}+S}X_{f,1} \\ &- r_{b,1}\left(1-\frac{X_{b,1}}{X_{b,max}}\right)X_{f,1} - DX_{f,1} + D_{sl,1}X_{b,1}\end{aligned} \tag{59c}$$

$$\begin{aligned}\frac{\partial X_{f,2}}{\partial t} = &\left(1-p\left(1-\frac{X_{b,2}}{\varepsilon X_{b,1}}\right)\right)\frac{\mu_{b,2}S}{K_{b,2}+S}X_{b,2} + \frac{\mu_{f,2}S}{K_{f,2}+S}X_{f,2} \\ &- r_{b,2}\left(1-\frac{X_{b,2}}{\varepsilon X_{b,1}}\right)X_{f,2} - DX_{f,2} + \left(D_{sl,1}+D_{sl,2}\right)X_{b,2}\end{aligned} \tag{59d}$$

$$\frac{\partial S}{\partial t} = D\left(S_{in}-S\right) - \sum_{i=1}^{2} V_{b,i}\frac{S}{K_{f,i}+S}X_{b,i} + V_{f,i}\frac{S}{K_{f,i}+S}X_{f,i}\,. \tag{59e}$$

Analytical treatment of steady state has not yet been performed.

3.13 "Quorum sensing"

Recent research has shown that bacteria can regulate gene expression at a population level through rapidly diffusing signal molecules. These molecules can be treated in a similar framework as secretion inhibitory or stimulatory substances (but now directed against the own species), especially if allosteric inhibition is used to switch abruptly from one kind of behaviour to another. Before any systematic analysis of this family of interactions can take place, a more thorough knowledge of this phenomenon is needed.

3.14 Pathway clearing for strict oligotrophs by copiotrophs

Basically this is a form of pure food competition, but one with a twist. Suppose we have two species of bacteria, both competing for the same limiting substrate, one with a low specific

affinity but high specific growth rate (the copiotroph), and one with a high specific affinity and low maximum specific growth rate, and which is inhibited at high substrate concentrations (the strict oligotroph). This inhibition may take many forms, but here we will follow Tan *et al.* [1996] who propose a form of competitive inhibition by the substrate itself. The differential equations become

$$\frac{\partial X_1}{\partial t} = Y_S V_{max,1} \frac{S}{K_{S,1} + S + S^2/K_{inh}} X_1 - DX_1 , \tag{60a}$$

$$\frac{\partial X_2}{\partial t} = Y_S V_{max,2} \frac{S}{K_{S,2} + S} X_2 - DX_2 , \tag{60b}$$

$$\frac{\partial S}{\partial t} = D(S_{in} - S) - V_{max,1} \frac{S}{K_{S,1} + S + S^2/K_{inh}} X_1 - V_{max,2} \frac{S}{K_{S,2} + S} X_2 . \tag{60c}$$

The single species equilibrium for the oligotroph becomes

$$S = \frac{K_{inh}}{2D}\left(Y_S V_{max,1} - D \pm \sqrt{\left(Y_S V_{max,1} - D\right)^2 - 4D^2 \frac{K_{S,1}}{K_{inh}}} \right), \tag{61a}$$

$$X_1 = Y_S (S_{in} - S). \tag{61b}$$

The lower root (if positive) is the stable equilibrium for *S*. The larger root is always unstable. Suppose the equilibrium is stable, and that *D* is small compared to the maximum growth rate of the oligotroph. If the input substrate level S_{in} is large enough, the growth rate at that level may be less than the dilution rate *D*:

$$Y_S V_{max,1} \frac{S_{in}}{K_{S,1} + S_{in} + S_{in}^2/K_{inh}} < D . \tag{62}$$

This can be the case if the larger root in (61a) is smaller than S_{in}. In that case we have three equilibria: (i) one stable (trivial) equilibrium with no bacteria, (ii) one unstable equilibrium with a small number of bacteria, and (iii) one stable with a high number of bacteria.

The action of the copiotrophs may now become clear. The single species equilibrium for them is

$$S = \frac{DK_{S,2}}{Y_S V_{max,2} - D}, \tag{63a}$$

$$X_2 = Y_S\left(S_{in} - S\right). \tag{63b}$$

Since the D is smaller than the maximum growth rate of the copiotroph (which is larger than that of the oligotroph), and no inhibition occurs, the trivial equilibrium becomes unstable against invasion of the copiotroph, because stable and unstable equilibria always alternate. If we assume that D is much smaller than the maximum growth rate for both species, the two single species equilibrium concentrations of substrate become:

$$S \approx \frac{DK_{S,1}}{Y_S V_{max,1}} = \frac{D}{a^0_{S,1}}, \quad \text{and} \quad S \approx \frac{DK_{S,2}}{Y_S V_{max,2}} = \frac{D}{a^0_{S,2}}. \tag{64}$$

Because the specific affinity of the oligotroph is higher than that of the copiotroph, the "only copiotroph" equilibrium becomes unstable against invasion by oligotrophs in its turn.
Because the gut ecosystem has a low dilution rate, and high input substrate level, this interaction may be important for the order of colonization and long term dynamics in the gut.

4. Discussion

In this report we have developed sets of differential equations for different types of interactions which may occur in the gut. Some of them, like pure food competition and predator prey interactions, are well-known interactions, but others such as the decoy effect, and pathway clearing by copiotrophs in high input substrate level/low dilution-rate environment are completely new. Others, such as binding competition and bacteriocins have received some attention in the literature, but improved formalisms have been used to describe them. Having a good analytical understanding of these interactions is essential both in interpreting the results of simulations, and in debugging the large scale models. If the simulations show totally different behaviour from the analytical solutions in steady state, the presence of one or more bugs must be suspected.

Further work is needed to solve the remaining differential equations analytically. Even so, those sets of differential equations which have been solved already allow a wealth of interactions to be modelled.

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